Anti-peptide Antibody Responses in Patients with Ataxia-telangiectasia

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Abstract

Background/objectives: Ataxia-telangiectasia (AT) is a rare inherited disorder caused by mutations in the ATM (Ataxia Telangiectasia Mutated) gene. Antibody response to diphtheria and tetanus toxoid vaccines may reveal indirect information about both cellular and humoral arms of the immune system in these patients. This study, therefore, set out to assess the specific antibody responses against tetanus and diphtheria vaccination among AT patients.

Methods: Thirty-eight AT patients were entered the study and an appropriate questionnaire was completed for all of them. Laboratory findings including alpha fetoprotein, lymphocyte subsets, serum immunoglobulin levels of IgG, IgG subsets, IgA, IgM, IgE and antibody response against diphtheria and tetanus toxoids were measured.

Results: Thirty-eight A-T patients were enrolled in this study. Based on the anti-tetanus and anti-diphtheria antibody production, 24 and 14 patients were categorized in responder (R) and non-responder (NR) groups, respectively. Respiratory tract infection was the most common infectious complication reported more frequently in the R comparing to NR group. Within the non-infectious manifestations, after cerebellar ataxia, ocular telangiectasia (52.6%) and FTT (26.3%) were the most frequent. 34.8% of individuals in R group but none of the NR patients had normal serum immunoglobulin profile (P=0.015). Contrarily, HIGM phenotype was found more frequent in NR group comparing to R group (50% vs. 17.4%, p= 0.063).

Conclusions: In accordance with the previous studies, we observed sufficient antibody response to diphtheria and tetanus vaccines in most of the AT patients.

Keywords Ataxia telangiectasia, immune deficiency, specific antibody response, anti-peptide antibody, polypeptide vaccine, humoral immune defect

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Introduction

Ataxia-Telangiectasia (A-T) is a rare inherited disorder caused by mutations in the ATM (Ataxia Telangiectasia Mutated) gene [1]. A-T is characterized by early onset of progressive cerebellar ataxia, oculocutaneous telangiectasia, endocrine disorders, increased radiosensitivity, predisposition to lymphoid malignancies, growth failures as well as immunodeficiency [2, 3].

Multiple causes predispose these patients to sinopulmonary infections that if left untreated may cause the development of bronchiectasis and pulmonary fibrosis, and eventually lead to pulmonary insufficiency and death4-6 ,2]].

Immunodeficiency in AT is associated with low number of T and B cells, defective B cell signaling, decreased immunoglobulins including IgA, IgE and/or IgG2 and raised levels of IgM in a subgroup of patients with defects in class-switch recombination [7-9]. Antibody response to diphtheria and tetanus toxoid vaccines may reveal indirect information about both humoral and cell-mediated arms of the immune system. While some research has been carried out on polysaccharide antibody production [10, 11], few empirical studies have done on anti-peptide antibody responses in patients with A-T. Thus, there is no enough evidence on specific antibody response to peptide vaccines.

This study was designed to evaluate specific antibody responses against tetanus and diphtheria vaccination among patients with A-T.

Materials and Methods
Patients

38 patients with A-T referred to immunology department of Children's Medical Center, Tehran, Iran were enrolled in the study. Diagnosis of A-T was done based on the criteria presented by the European Society for Immunodeficiency (ESID) and the Pan-American Group for Immunodeficiency (PAGID) in all cases [12, 13]. An appropriate questionnaire was developed to collect the necessary information including age, sex, first clinical presentation, age at onset of symptoms, age at time of diagnosis, diagnostic delay, years of follow up, consanguinity of parents, past medical history, immunological data, history of other complications including recurrent infections, autoimmunity, allergy, organomegaly, lymphadenopathy, malignancy, nystagmus, conjunctivitis, otitis, neurodevelopmental delay and gastrointestinal manifestations. This questionnaire was completed for all patients included in the study using the recorded data in Iranian PID registry (IPIDR).

Laboratory information

Laboratory findings including alpha fetoprotein (AFP) and lymphocyte subsets were measured for patients using Electrochemiluminescence and flow cytometry (Partec PAS, Munster, Germany), respectively. Moreover, serum immunoglobulin levels of IgG, IgA, and IgM were measured using nephelometry (Behring Nephelometer, Behring werk, Marburg, Germany). Serum levels of IgE, IgG1, IgG2, IgG3, and IgG4 and antibody response against diphtheria and tetanus toxoids were measured using the Enzyme-Linked Immunosorbent Assay (ELISA).

Results
Demographic characteristic of patients with A-T

Patients with A-T included 14 male and 24 female with mean age of 9.5 (7.8-11.5) years old who were enrolled in this study. The patients were followed for 127.2 years. Parental consanguinity was present in 82.9% of cases (29 cases). Demographic data of all patients are summarized in **Table 1**. Patients with a diphtheria antitoxin titer of <0.01 IU/ml and a tetanus antitoxin titer of <0.1 IU/ml were considered as unprotected. Patients with A-T were categorized into responder (R) and non-responder (NR) groups according to the ability for producing specific anti-peptide antibody against mentioned vaccine. Based on the anti-tetanus and anti-diphtheria antibody production, 24 and 14 patients were categorized in R and NR groups, respectively. **Figure 1** shows that the first clinical

presentations included cerebellar ataxia (42% of cases), infections (40% of cases), ocular telangiectasia (5% of cases) and developmental delay in, 5% of patients with A-T, respectively.

Clinical manifestations of patients with A-T
Overall, cerebellar ataxia (84.2%) and infections (78.9%) were the most common clinical manifestations in patients with A-T, which there was no significant difference in the frequency of cerebellar ataxia and infections between R and NR groups. **Table 2** provides detailed information on clinical data for patients with A-T.

Respiratory tract infection was the most common infectious complication reported more frequently in the R group compared to NR group (15 cases (62.5%) vs. 8 cases (57.1%), p= 0.744) but it was not statistically significant.

Among the non-infectious manifestations, after cerebellar ataxia, ocular telangiectasia (52.6%) and failure to thrive (26.3%) were found as the most frequent manifestations. Ocular telangiectasia [12 cases (50%) vs. 8 cases (57.1%), p=0.671], was more prevalent in R group but the difference was not statistically significant. However, the frequency of failure to thrive was significantly higher in R group [9 cases (37.5%) vs. 1 case (7.1%), *p*= 0.05]. Hepatomegaly [2 cases (14.3%) vs. 0 case (0%), p=0.129] and splenomegaly [3 cases (21.4%) vs. 0 case (0%), p= 0.043] were found to be more frequent in NR group than patients in R group.

Table 1. Demographic data of patients with A-T

Demographic value	Total number of patients with A-T	Responder group	Non-Responder group	P- value
Number of patients (%)	38	24 (63.2)	14 (36.8)	-
Sex (male/female)	14/24	9/15	5/9	0.912
Consanguinity (%)	29 (82.9)	17 (70)	12 (85.7)	0.377
First consanguinity (%)	20 (58.8)	10 (41.6)	10 (71.4)	0.092
Age at time of study; years, mean (IQR)	9.5 (7.87-11.5)	9.25 (8-11.5)	9.5 (6-12)	0.726
Age at diagnosis; years, mean(IQR)	6 (4-8)	6.1 (2.45-8)	6 (4.5-9.54)	0.549
Age at onset of disease; years, mean (IQR)	1 (0.75-1)	1 (0.59-1.15)	1 (1-1.25)	0.355
Age at onset of cerebellar ataxia; years, mean (IQR)	1.25 (1-3)	1.8 (1-4)	1 (1-2.25)	0.089
Age at onset of ocular telangiectasia; years, mean (IQR)	4 (2.5-6)	5 (3-7)	3.15 (2-5.5)	0.125
Age at onset of infection; years, mean (IQR)	1 (1-4.5)	2 (1-5.5)	1 (1-3)	0.567
Diagnostic delay; years, mean (IQR)	4.37 (1.90-6.68)	4.5 (1.21-6.75)	4.25 (3.33-8.29)	0.366
Years of follow up; mean (IQR)	3.5 (1-5.75)	5 (1.85-6.72)	2 (0.29-4.5)	0.025*

The mean is shown with 25th and 75th percentiles.
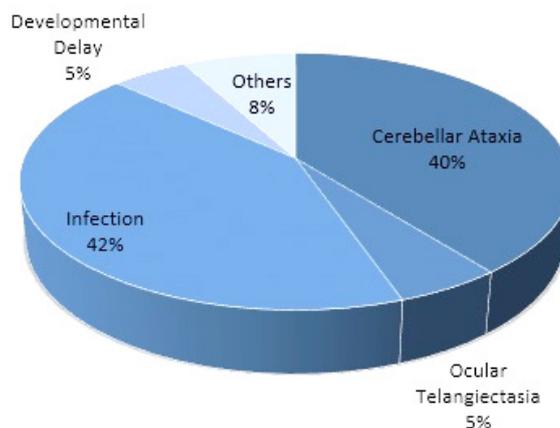* P-value of <0.05is considered as statistically significant

Figure 1. First clinical presentations in patients with A-T

Immunological features of patients with A-T

Table 3 provides the detailed results obtained from the preliminary analysis of the immunological values. The mean serum level of AFP was equal to 246 ng/dL (99.95-622). AFP level elevated in both groups, but patients in R group [246 (132.5-440.57) ng/dL] showed a higher level of this marker compared to the NR group [199.5 (90.5-626.75) ng/dL; *p*=0.640]. A decreased frequency of CD3+, CD4+ T cells and of B cells was reported in 28.6%, 57.1%, and 83.3% of patients with A-T, respectively. Respiratory tract infection was more frequent in patients with CD4+ T cell deficiency than patients with normal CD4+ T cell count (p = 0.02).

There was no significant correlation between lymphocyte subset abnormality and antibody response to vaccines. According to serum Ig profile, immunologic phenotyping was compatible with IgAD in 24.3% of cases (n=9), normal Ig level in 21.6% of cases (n=8), hypogammaglobulinemia in 24.3% of cases (n=9), and Hyper IgM Syndrome (HIGM) in 29.7% of cases (n=11). 34.8% of patients in R group had normal serum immunoglobulin profile, but none of the NR patients had normal serum immunoglobulin profile (P=0.015). Contrarily, HIGM phenotype was found to be more frequent in NR group compared to R group (50% vs. 17.4%, p= 0.063). Higher total serum IgG concentration was reported in R group compared to NR group [637 (309.75-933) mg/dL vs. 297.5 (51-950.5) mg/dL, p= 0.446]. Serum IgG_2 subclass was also found to be higher in R group [60 (46.5-147.5)] compared to NR group [30.5 (23-51.25)] (P = 0.007). The mean of serum IgA was observed to be lower in patients with history of sinusitis [7.5 (1.0-56.2) vs. 18.0 (5.0-87.2)] and chronic diarrhea [7(0.0-63.0) vs. 17.0 (5.0-84.5)] than patients who had no such history, however, the differences were not statistically significant.

Table 2. Clinical manifestations of patients with A-T

Clinical manifestation	Total number of patients with A-T	Responder group	Non-Responder group	P- value
Cerebellar ataxia (%)	32 (84.2)	20 (83.3)	12 (85.7)	1.0
Ocular telangiectasia (%)	20 (52.6)	12 (50)	8 (57.1)	0.671
Infections (%)	30 (78.9)	19 (79.2)	11 (78.6)	1.0
Respiratory tract infection (%)	23 (60.5)	15 (62.5)	8 (57.1)	0.744
URI (%)	13 (34.2)	9 (37.5)	4 (28.6)	0.728
Sinusitis (%)	8 (21.1)	7 (29.2)	1 (7.1)	0.216
Sinopulmonary infection (%)	17 (44.7)	13 (54.2)	4 (28.6)	0.126
Pneumonia (%)	16 (42.1)	10 (41.7)	6 (42.9)	0.943
Otitis media (%)	8 (21.1)	4 (16.7)	4 (28.6)	0.433
Mucocutaneous infection (%)	5 (13.2)	4 (16.7)	1 (7.1)	0.633
Diarrhea (%)	7 (18.4)	5 (20.8)	2 (14.3)	1.0
Other GI manifestations (%)	7 (18.4)	5 (20.8)	2 (14.3)	1.0
Lymphadenopathy (%)	1 (2.6)	1 (4.2)	0 (0)	1.0
Hepatomegaly (%)	2 (5.3)	0 (0)	2 (14.3)	0.129
Splenomegaly (%)	3 (7.9)	0 (0)	3 (21.4)	0.043*
Nystagmus (%)	5 (13.2)	4 (16.7)	1 (7.1)	0.633
Conjunctivitis (%)	2 (5.3)	2 (8.3)	0 (0)	0.522
Autoimmunity (%)	4 (10.5)	2 (8.3)	2 (14.3)	0.616
Allergy (%)	4 (10.5)	4 (16.7)	0 (0)	0.276
FTT (%)	10 (26.3)	9 (37.5)	1 (7.1)	0.059
Fever (%)	8 (21.1)	4 (16.7)	4 (28.6)	0.433

URI: Upper Respiratory tract Infection; GI: Gastrointestinal; FTT: Failure To Thrive
* P-value of <0.05is considered as statistically significant.

Table 3. Laboratory findings of patients with A-T

Laboratory value	Total number of patients with A-T	Responder group	Non-Responder group	P- value
IgG (mg/dl)	622 (133.25-936.5)	637 (309.75-933)	297.5 (51-950.5)	0.446
IgG1 (mg/dl)	661.5 (443-770.5)	534 (389-762)	761 (743.5-916)	0.085
IgG2 (mg/dl)	52 (33-103)	60 (46.5-147.5)	30.5 (23-51.25)	0.007*
IgG3 (mg/dl)	54 (34-74)	54 (36.5-71.5)	48.5 (25-85)	0.792
IgG4 (mg/dl)	8.4 (3.2-19)	6.2 (2.9-17.5)	11 (4.5-31)	0.330
IgA (mg/dl)	13.5 (2.5-78)	10 (4-75)	14 (1-93.5)	0.895
IgM (mg/dl)	144 (85-223)	144 (85-204)	154.5 (93-569.5)	0.415
IgE (mg/dl)	5.0 (1.0-10.0)	5.7 (1.0-19.0)	1.7 (1-6.25)	0.158
WBC (cell/μL)	5665 (3960-7502)	4995 (3797.5-6837.5)	7125 (4185-8725)	0.183
Lymphocyte (cell/μL)	1515 (1218-2489)	1490 (1021-2334)	1688 (1244.5-4275.1)	0.405
PMN (cell/μL)	2836 (2254-5030)	2600 (2167-3992.5)	3976.5 (2118.2-5382.7)	0.363
CD3+ (% of lymphocytes)	64.0 (51-66)	62.0 (53.25-64)	66.0 #	0.476
CD4+ (% of lymphocytes)	29.0 (20-46)	24.5 (20-30.5)	46.0 #	0.154
CD8+ (% of lymphocytes)	22.0 (17-38)	23.5 (16.75-34.75)	22.0 #	0.858
CD19+ (% of lymphocytes)	8.5 (4-13)	5.75 (3.22-16)	10.5 #	0.355
CD16+56+ (% of lymphocytes)	20.2 (12.5-258)	15.0	256.5 #	0.248
Serum AFP level (IU/mL)	246 (99.95-622)	246 (132.5-440.57)	199.5 (90.5-626.75)	0.640
Plt ()	338 (293-397)	349.5 (304.7-414.7)	338 (150-401)	0.375
Hb (g/dl)	12.6 (11.2-13.4)	12.6 (11.35-13.3)	12.4 (10.25-13.6)	0.824

AFP: Alpha Feto Protein; PMN, Polymorphonuclear cells; WBC: White Blood Cells; Hb: Hemoglubin; Plt: Platelet
* P-value of <0.05 is considered as statistically significant.
# representing that reported only for one patient

Discussion

A-T is a rare autosomal recessive multisystem disorder caused by mutations in the ATM gene located on chromosome 11.[1] ATM gene plays a critical role in DNA double strand break recognition and/or repair during CSR and also V(D) J recombination[15, 16]. Typically, the more frequent type of immunodeficiency found in patients with A-T is classic (early onset) type and it often emerges as a result of antibody deficiency. In patients with A-T, the occurrence of deficiency in serum level of IgA, IgG2, IgG4, and IgE is common. A minority of these patients may also show increased serum level of IgM, which may be misdiagnosed as HIGM syndrome[17-19].

In the present study, immunologic phenotyping included compatible with normal Ig level in 21.6% of cases, IgA deficiency in 24.3% of cases, hypogammaglobulinemia in 24.3% of cases, and HIGM in 29.7% of cases. In the recent report by Ghiasy et al. in 2017, the immunologic phenotyping in patients with A-T included normal immunoglobulin level (22.8%), IgA deficiency (37.9%) hypogammaglobulinemia (18.1%) and HIGM profile in 21.2% of cases[20]. Nevertheless, in the present study, HIGM was found to be the most prominent humoral immune defect. In the present study, patients in R group had normal Ig level; whereas, HIGM was found to be the most prevalent immunologic phenotype in NR patients (50%) followed by hypogammaglobulinemia (28.6%), IgA deficiency (21.4%), and normal Ig level (0%). Taken together, these findings suggest that patients with A-T having HIGM probably show more ineffective response to peptide vaccines.

In the current study, no correlation was found between lymphocyte subsets and anti-peptide antibody response, but the susceptibility to respiratory tract infection was observed more frequently in patients with CD4+ T cell deficiency.

According to the findings of the study by Driessen *et al*, this is mostly resulted from reduced B- and T-cell production related to disturbed V (D)J recombination, and consequently due to limited repertoire of B-cell and T-cell receptor. However, despite of reduced naive T-cell numbers, patients with A-T have normal numbers of circulating effector CD4+ and CD8+ T cells indicating normal terminal differentiation. Therefore, they mainly have antibody deficiency rather than T-cell deficiencies [9]. This finding is consistent with those of previous studies showing that there is no correlation between antibody production and susceptibility to infection in patients with A-T. Albeit the severity of infections tend to be related to low levels of serum IgA and IgG and even to specific antibody response [3, 10, 21]. The results of the present study showed that the occurrence of infections was more frequent in non-responder patients; however, a statistically significant relationship was not found, due to few number of patients.

In the past three decades, a number of researches have carried out to determine the specific antibody response in patients with A-T. For example, the results of the study conducted by Weemaes et al. showed defective primary IgG, IgM and IgA antibody responses to T cell dependent antigens but normal secondary responses were found to diphtheria, tetanus and polio vaccine22]]. Another research also reported normal anti-tetanus toxoid antibody level after booster immunization in 12 patients with A-T [10]; whereas in another study, weak antibody response to tetanus and polio antigen have been reported [23]. Interestingly, the native CRM197 used in the conventional diphtheria vaccines was observed to induce lower antibody levels than the diphtheria toxoid [24, 25]. Pederson *et al*. in their study showed sufficient antibody response to diphtheria and tetanus vaccines in about 81% of patients with A-T [26]. Similar results were obtained in another study showing protective anti-tetanus

levels in 97% of cases and anti-diphtheria levels in 94% of cases. But the results of previous studies showed no association between predisposition to respiratory tract infections and the presence of IgG, IgA, or IgG subclass deficiency [17]. A retrospective cohort study also found protective antibody titers in most of the patients at baseline and during the follow up. However, the tetanus-specific IgG levels fell from protective to non-protective levels for 2 patients during the course of follow-up [5]. Further researches suggested pre-existing protective antibody level to diphtheria and tetanus as an indicator of sufficient anti-peptide antibody response [11, 27].

In this research, sufficient antibody response to diphtheria and tetanus vaccines was observed in 24 patients, while 14 patients ineffectively responded to the mentioned vaccines. This finding was in accordance with those of previous studies in which most of the patients reported to be responder. However, the difference in the number of patients between responder and non-responder groups was found to be more obvious in the present study. Thus, future studies are suggested to be carried out with more sample size and revaccination of patients with A-T during the course of the study.

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